7

32-95

PEPTIDE-ENHANCED CATIONIC LIPID TRANSFECTIONS

Field of the Invention

Compositions of peptides, optionally conjugated to DNA-binding groups, and cationic lipids useful for transfecting eukaryotic cells are disclosed. Also disclosed are methods of transfecting eukaryotic cells employing such compositions.

Background of the Invention

Lipid aggregates such as liposomes can function to facilitate introduction of macromolecules, such as DNA, RNA, and proteins, into living cells. Lipid aggregates comprising cationic lipid components can be effective for delivery and introduction of large anionic molecules, such as nucleic acids, into certain types of cells. See Felgner, P.L. and Ringold, G.M. (1989) Nature 337:387-388 and Felgner, P.L. et al. (1987) Proc. Natl. Acad. Sci. USA 84:7413. Since the membranes of most cells have a net negative charge, anionic molecules, particularly those of high molecular weight, are not readily taken up by cells. Cationic lipids aggregate to and bind polyanions, such as nucleic acids, tending to neutralize the negative charge. The effectiveness of cationic lipids in transfection of nucleic acids into cells is thought to result from an enhanced affinity of cationic lipid-nucleic acid aggregates for cells, as well as the function of the lipophilic components in membrane fusion.

Cationic lipids are not universally effective for transfection of all cell types. Effectiveness of transfection of different cells depends on the particular cationic lipid composition and the type of lipid aggregate formed. In general,

polycationic lipids are more efficient than monocationic lipids in transfecting eukaryotic cells. Behr, J-P. et al. (1989) Proc. Natl. Acad. Sci. 86:6982-6986, Hawley-Nelson, P., et al. (1993) FOCUS 15:73 and U.S. Patent No. 5,334,761 (Gebeyehu et al.). Behr et al. and EPO published application 304 111 (1990), for example, describe improved transfection using carboxyspermine-containing cationic lipids including 5-carboxyspermylglycine dioctadecyl-amide (DOGS) and dipalmitoylphosphatidylethanolamine 5-carboxyspermylamide (DPPES). Despite their relative effectiveness, however, successful transfection of eukaryotic cell cultures using polycationic lipid reagents requires high dosages of nucleic acid (approximately 10^5 DNA molecules per cell).

Many biological materials are taken up by cells by receptor-mediated endocytosis. See: Pastan and Willingham (1981) Science 214:504-509. This mechanism involves binding of a ligand to a cell-surface receptor, clustering of ligand-bound receptors, and formation of coated pits followed by internalization of the ligands into endosomes. Both enveloped viruses, like influenza virus and alphaviruses, and non-enveloped viruses, like adenovirus, infect cells via endocytotic mechanisms. See: Pastan, I. et al. (1986) in Virus Attachment and Entry into Cells, (Crowell, R.L. and Lonberg-Holm, K., eds.) Am. Soc. Microbiology, Washington, p. 141-146; Kielian, M. and Helenius, A. (1986) "Entry of Alphaviruses" in The Togaviridae and Flaviviridae, (Schlesinger, S. and Schlesinger, M.J., eds.) Plenum Press, New York p.91-119; FitzGerald, D.J.P. et al. (1983) Cell 32:607-617.

The introduction of foreign DNA sequences into eukaryotic cells mediated by viral infection is generally orders of magnitude more efficient than transfection with cationic lipid reagents. Viral infection of cell cultures requires fewer than 10 virus particles per cell. Although the detailed mechanism of fusion is not fully understood and varies among viruses, viral fusion typically involves specific fusagenic agents such as viral

proteins, viral spike glycoproteins and peptides of viral spike glycoproteins. Vesicular stomatitis virus (VSV) fusion, for example, is thought to involve interaction between the VSV glycoprotein (G protein) and membrane lipids (Schlegel, R. et al. (1983) Cell 32:639-646). The VSV G protein reportedly binds preferentially to saturable receptors such as acidic phospholipid phosphatidylserine (Schlegel, R. and M. Wade (1985) J. Virol. 53(1):319-323). Fusion of influenza virus involves hemagglutinin HA-2 N-terminal fusagenic peptides. See Kamata, H. et al. (1994) Nucl. Acids Res. 22(3):536-537.

The efficiency of cationic lipid transfections has recently been shown to be enhanced by the addition of whole virus particles to the transfection mixture. See Yoshimura et al. (1993) J. Biol. Chem. 268:2300. Certain viral components may also enhance the efficiency of cationic lipid-mediated transfection. See U.S. Patent Application Serial No. 08/274,397, filed July 12, 1994, incorporated by reference in its entirety herein. The use of peptides from viral proteins to enhance lipid-mediated transfections was also recently suggested by Kamata et al. (1994) Nucl. Acids Res. 22:536. Kamata et al. suggest that LIPOFECTIN™-mediated transfections may be enhanced 3-4-fold by adding influenza virus hemagglutinin peptides to the transfection mixture. Despite these positive early indications, results vary as to the effectiveness of including fusagenic peptides in lipidic transfection compositions. Remy et al. (1995) Proc. Natl. Acad. Sci. USA 92:1744 report that "[a]ddition of lipids bearing a fusagenic or a nuclear localization peptide head group to the (polycationic lipid-DNA complex) particles does not significantly improve an already efficient system."

The present invention is based on the discovery that peptide sequences from viral proteins can significantly enhance the efficiency of cationic lipid-mediated transfection of eukaryotic cells. The compositions and methods of the invention comprise fusagenic or nuclear localization peptides which significantly improve the efficiency of transfection when bound to nucleic acid

prior to adding the transfection reagent. These fusagenic and nuclear localization peptides form a noncovalent association or complex with the DNA. Complex formation may be enhanced by covalently coupling the peptide to a DNA binding group, which binds to the nucleic acid through conformational or charge interactions between the binding group and the DNA. These bound nucleic acids are more efficiently transported to the cell nucleus, thus requiring less nucleic acid starting material. The cationic lipid compositions of the present invention provide significant advantages over prior art compositions, including enhanced transformation frequency.

Summary of the Invention

The present invention provides compositions and methods for transfecting eukaryotic cells, particularly higher eukaryotic cells, with nucleic acids. Nucleic acids, both DNA and RNA, are introduced into cells such that they retain their biological function. A composition for transfecting eukaryotic cells comprising a peptide-nucleic acid complex and a cationic lipid is provided. Transfecting compositions comprise a fusagenic or nuclear localization peptide which binds nucleic acid. This peptide-nucleic acid complex is subsequently combined with cationic lipid to form a peptide-nucleic acid-lipid aggregate which facilitates introduction of the anionic nucleic acid through cell membranes, including the nuclear membrane.

Peptides useful in transfection compositions include peptides of viral fusagenic proteins, peptides of viral nuclear localization signals, and other naturally occurring and synthetic fusagenic and nuclear localization peptides. Transfecting compositions comprising viral peptides of influenza virus, vesicular stomatitis virus and simian virus 40 are of particular interest. Transfecting compositions comprising peptides of viral proteins conjugated to DNA-binding groups (modified peptides) are also of interest.

Inclusion of a peptide- or modified peptide-nucleic acid complex in a cationic lipid transfection composition significantly enhances transfection (2-fold or more) compared to transfection mediated by the cationic lipid alone. Enhancement of transfection by peptides or modified peptides is pronounced in a wide variety of cell lines, including human primary cell lines.

Monovalent or preferably polyvalent cationic lipids are employed in transfecting compositions. Preferred polyvalent cationic lipids are lipospermines, specifically DOSPA (2,3-droleylocy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate). Cationic lipids are optionally combined with non-cationic lipids, particularly neutral lipids, for example lipids such as DOPE (dioleoylphosphatidyl-ethanolamine). A cationic lipid composition composed of a 3:1 (w/w) mixture of DOSPA and DOPE is generally useful in transfecting compositions of this invention. Preferred transfection compositions are those which induce substantial transfection of a higher eukaryotic cell line.

The methods of the present invention involve contacting a eukaryotic cell with a transfecting composition comprising a fusagenic or nuclear localization peptide, optionally conjugated to a DNA-binding group, wherein said peptide or modified peptide is non-covalently associated with the nucleic acid. This peptide-nucleic acid complex is then combined with a cationic lipid. A preferred method employs a viral peptide of influenza virus, vesicular stomatitis virus or simian virus 40. Methods of this invention are applicable to transfection of adherent or suspension cell lines, in general to animal cell lines, specifically to mammalian, avian, reptilian, amphibian and insect cell lines and more specifically to animal primary cell lines, human primary cell lines, stem cell lines, and fibroblasts.

In one alternative transfection method, a fusagenic peptide is conjugated to a DNA-binding group to produce a modified

peptide which is then bound to the nucleic acid to be introduced into the cell. The modified peptide-nucleic acid complexes are then admixed with cationic lipid and the resulting mixture is employed to transfect cells.

In a second alternative transfection method, a fusagenic or nuclear localization peptide is bound to nucleic acid. The peptide-nucleic acid complex is then admixed with cationic lipid and the resulting mixture is employed to transfect cells.

The transfection methods of the present invention can be applied to in vitro and in vivo transfection of eukaryotic cells, particularly to transfection of higher eukaryotic cells including animal cells. The methods of this invention can be used to generate transfected cells which express useful gene products. The methods of this invention can also be employed as a step in the production of transgenic animals. The methods of this invention are useful as a step in any therapeutic method requiring introduction of nucleic acids into cells. In particular, these methods are useful in cancer treatment, in in vivo and ex vivo gene therapy, and in diagnostic methods. The transfection compositions of this invention can be employed as research reagents in any transfection of eukaryotic cells done for research purposes. Nucleic acids that can be transfected by the methods of this invention include DNA and RNA from any source comprising natural bases or non-natural bases, and include those encoding and capable of expressing therapeutic or otherwise useful proteins in cells, those which inhibit undesired expression of nucleic acids in cells, those which inhibit undesired enzymatic activity or activate desired enzymes, those which catalyze reactions (ribozymes), and those which function in diagnostic assays.

The compositions and methods provided herein can also be readily adapted in view of the disclosure herein to introduce biologically-active macromolecules other than nucleic acids including, among others, polyamines, polyamine acids,

polypeptides and proteins into eukaryotic cells. Other materials useful, for example as therapeutic agents, diagnostic materials, research reagents, which can be bound to the peptides and modified peptides and introduced into eukaryotic cells by the methods of this invention.

All publications and patents referred to herein are specifically incorporated by reference in their entirety.

Brief Description of the Figures

Figure 1 is a bar graph showing enhancement of transfection of human fibroblast cells with various peptides added to LIPOFECTAMINE™-DNA transfection mixtures.

Figure 2 is a graph showing enhancement of transfection of human fibroblasts by including varying concentrations of spermine-NLS and spermine-VSVG complexed to DNA prior to addition of LIPOFECTAMINE™.

Figure 3 is a bar graph showing enhancement of transfection of human fibroblasts by including a combination of DNA with spermine, mixtures of spermine and peptide-DNA complexes and various peptide-DNA complexes.

Figure 4 is a bar graph showing the effect of order of addition of reagents on enhancement of transfection of human fibroblast cells.

Figure 5 is a bar graph showing enhancement of transfection of human fibroblasts by including NLS-DNA and poly-L-lysine-DNA complexes in LIPOFECTAMINE™.

Figure 6 is a bar graph showing the effect of concentration of spermine-NLS-DNA complexes on enhancement of transfection of BHK-21 cells.

Figure 7 is a bar graph showing the effect of concentration of spermine-VSVG-DNA complex on enhancement of transfection of BHK-21 cells.

Detailed Description of the Invention

The present invention provides improved methods for transfecting eukaryotic cells with nucleic acids by employing peptides and cationic lipids. The improvement relates to the use of a peptide-nucleic acid complex to enhance the efficiency of cationic lipid-mediated transfection. This invention has significant advantages over prior art methods of transfection which employ cationic lipids. The fusagenic or nuclear localization peptides of the invention, optionally covalently coupled to a DNA-binding group or polyamine, form a complex with the DNA. These complexed nucleic acids are more efficiently transported to the cell nucleus, thus enhancing the efficiency of cationic lipid-mediated cell transfection. Because of the improved efficiency of transfection, considerably less nucleic acid is required for effective transfection. The transfection compositions of the invention, by virtue of complex formation between the nucleic acid and peptide, provide enhanced transfection as compared to prior art cationic lipid transfection compositions.

The following definitions are employed in the specification and claims.

The term "transfection" is used herein generally to mean the delivery and introduction of biologically functional nucleic acid into a cell, i.e. a eukaryotic cell, in such a way that the nucleic acid retains its function within the cell. The term transfection includes the more specific meaning of delivery and introduction of expressible nucleic acid into a cell such that the cell is rendered capable of expressing that nucleic acid. The term expression means any manifestation of the functional presence of the nucleic acid within a cell, including both

transient expression and stable expression. Nucleic acids include both DNA and RNA without size limits from any source comprising natural and non-natural bases. Nucleic acids can have a variety of biological functions. They may encode proteins, comprise regulatory regions, function as inhibitors of gene or RNA expression (e.g., antisense DNA or RNA), function as inhibitors of proteins, function to inhibit cell growth or kill cells, catalyze reactions or function in a diagnostic or other analytical assay.

Transfection efficiency is "enhanced" when an improvement of at least about 5 percent, preferably about 10 percent, and more preferably about 20 percent in efficiency is shown using the protocols set forth in the examples hereof.

The term "DNA-binding group" is used herein generally to mean a protein, peptide, polypeptide or polyamine which is capable of associating with nucleic acids. Although the mechanism of association depends upon the particular binding group, sequence specificity generally results from an ensemble of mutually favorable interactions between a binding group and its target DNA. Some DNA-binding groups, for example, interact with the DNA's paired bases and sugar-phosphate chains through direct contacts, including hydrogen bonds, salt bridges and van der Waals forces. Other groups function through sequence-specific conformational variations in DNA rather than from sequence-specific hydrogen bonding interactions between DNA and protein. It will be understood that the term "DNA-binding group" includes any protein, peptide, polypeptide or polyamine which is capable of binding nucleic acid, without regard to the mechanism of binding. DNA-binding groups are known to the art and widely available in commerce.

"Peptide" is a generic term which includes straight chain and branched peptides of all types, both naturally occurring and synthetic, without regard to molecular weight or amino acid composition.

The term "modified-peptide" is used herein generally to mean a peptide which has been chemically modified to include a DNA-binding group covalently attached thereto. The term "modified-peptide" as used herein includes "polyamine-peptide conjugate" wherein the covalently attached DNA-binding group is a polyamine, "spermine-modified peptide" wherein the DNA-binding group is spermine, and "modified-nuclear localization" peptides, wherein the DNA-binding group is a nuclear localization peptide.

The term "peptide-nucleic acid complex" generally refers to the noncovalent association between a peptide and a nucleic acid. As used herein, a "peptide-nucleic acid complex" is formed prior to the addition of cationic lipid to the transfection composition. The term "peptide-nucleic acid complex" includes both unmodified and modified peptides in combination with nucleic acid.

"Lipid aggregate" is a generic term that includes liposomes of all types both unilamellar and multilamellar as well as vesicles, micelles and more amorphous aggregates. A cationic lipid aggregate is a lipid aggregate comprising sufficient cationic lipid, optionally in combination with non-cationic lipids, such that the lipid aggregate has a net positive charge. Cationic lipids and lipid aggregates are capable of aggregating the peptide-nucleic acid complexes of the invention.

Transfection activity or efficiency is measured by detecting the presence of the transfected nucleic acid in a cell. This is often assessed by measuring the biological function of the nucleic acid in the cell, and most often assessed by measuring the level of transient or stable expression of a reporter gene comprised in the transfected nucleic acid. Reporter gene expression depends among other things on the amount of nucleic acid transfected as well as promoter function in the cell. Transfection activity can also be assessed by determining the percent of cells in a sample that have been transfected, for example, by assessing reporter gene expression using cell

counting or in situ staining methods. The transfection methods of this invention employing peptides in combination with cationic lipids can display significant enhancement of transfection (2-fold or more) over transfection methods employing comparable cationic lipids alone.

The method of this invention involves contacting a eukaryotic cell with a transfection composition comprising a peptide-nucleic acid complex (or a modified peptide-nucleic acid complex) and a cationic lipid. The transfection composition optionally comprises a non-cationic lipid, preferably a neutral lipid. The peptide is a fusagenic peptide of a viral protein and is preferably a fusagenic peptide of influenza virus or vesicular stomatitis virus. Also preferred is a nuclear localization signal peptide of simian virus 40, particularly the nuclear localization sequence of the SV40 large T antigen. Kalderon et al. (1984) Cell 39:499; and Lanford et al. (1986) Cell 46:575. Transfecting compositions comprising peptides of viral proteins conjugated to a DNA-binding group are particularly preferred. The DNA-binding group is a protein, peptide, polypeptide or polyamine capable of forming a noncovalent association with the base pairs of nucleic acid. Enhanced transfection methods of this invention have been demonstrated with the prototype nuclear localization signal peptide from simian virus 40 and the prototype fusagenic peptides from influenza (HApep; E5 and K5 amphiphilic peptides) and vesicular stomatitis virus (G protein). The DNA-binding group is a polyamine capable of forming a noncovalent association with the base pairs of the nucleic acid. Enhanced transfection methods of this invention have been further exemplified using the prototype DNA-binding group, spermine.

The fusagenic or nuclear localization peptides form a noncovalent association or complex with the nucleic acid. This peptide-nucleic acid complex forms as a consequence of conformational or charge interactions between the peptide and the base pairs of the DNA. A peptide-nucleic acid complex forms spontaneously in an appropriate medium.

The fusagenic or nuclear localization peptides, when covalently coupled to a DNA-binding group (modified-peptide), forms a noncovalent association or complex with the nucleic acid. This modified-peptide-nucleic acid complex forms as a consequence of conformational or charge interactions between the DNA-binding group and the DNA. For example, the prototype spermine-peptide-nucleic acid complex likely forms as a consequence of charge interactions between the amines of spermine and the phosphates on the DNA backbone. A modified-peptide-nucleic acid complex forms spontaneously in an appropriate medium.

The transfection solution containing the peptide-nucleic acid or modified-peptide-nucleic acid complexes may then be admixed with a cationic lipid, alone or in combination with a non-cationic lipid, to form a peptide-nucleic acid-lipid aggregate. A peptide-nucleic acid-lipid aggregate forms spontaneously in an appropriate medium or various well-known techniques may be also be employed to produce a desired type of lipid aggregate. The relative amounts of cationic lipid and non-cationic lipid employed depends on a number of factors, including the toxicity of the lipids to the cell and the environment (e.g. medium) in which the aggregate is to be employed. The kinds and amounts of lipids employed is typically balanced to minimize cell toxicity and maximize transfection efficiency.

Viral peptides can be isolated by a variety of well-known techniques, for example using the cationic detergent DTAB as described in Glushakova, S.E., et al. (1985) "Influenza viral glycoproteins isolation using cationic detergent dodecylmethylammonium bromide and its subsequent integration into liposomal membrane" Mol. Genet. Microbiol. Virol. 4:39-44. Alternatively, viral peptides can be produced by a variety of standard chemical syntheses methods. Viral fusagenic peptides, for example, can be synthesized using automated solid phase peptide synthesis as described, e.g., in Stewart et al. (1984) Solid Phase Peptide Synthesis, Pierce Chemical Company, Rockford, Illinois. Fusagenic peptides from influenza and vesicular

stomatitis virus, including the exemplified hemagglutinin peptide, K5 and E5 amphiphilic peptides and G protein, are particularly useful in the methods of this invention. Nuclear localization signal peptides from simian virus 40, including the exemplified NLS peptide, are also preferred.

Modified-peptides can be prepared by a variety of well-known coupling techniques, for example using a heterobifunctional cross-linking agent as described in the Examples hereof. A variety of cross-linking agents are known to the art and widely available in commerce including, without limitation, succinimidyl or maleimidyl cross-linkers, such as Sulfosuccinimidyl 4-(*p*-maleimidophenyl)butyrate (Sulfo-SMPB), disuccinimidyl suberate, succinimidyl 4-(*p*-maleimidophenyl)butyrate (SMPB), 4-succinimidyloxycarbonyl-α-methyl-α-(2-pyridyldithio)-toluene (SMPT), Sulfosuccinimidyl 6-[3-(2-pyridyldithio)propionamido]hexanoate (Sulfo-LC-SPDP), Succinimidyl 6-[3-(2-pyridyldithio)propionamido]hexanoate (LC-SPDP), N-Succinimidyl 3-(2-pyridyldithio)propionate (SPDP), Sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (Sulfo-SMCC), Succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC); *m*-Maleimidobenzoyl-N-hydroxysulfosuccinimide ester (Sulfo-MBS), *m*-Maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), Sulfosuccinimidyl(4-iodoacetyl)aminobenzoate (Sulfo-SIAB), N-Succinimidyl(4-iodoacetyl)aminobenzoate (SIAB). Methods for conjugating peptides and polyamines are well-known in the art. Representative methods are disclosed in Staros, J.V. (1982) Biochemistry 21:3990.

Media employed in transfections should preferably be free of components, like serum or high salt levels, that can inhibit cationic lipid-mediated transfection of cells.

A variety of cationic lipids is known in the art. Generally, any cationic lipid, either monovalent or polyvalent, can be used in the compositions and methods of this invention. Polyvalent cationic lipids are generally preferred. Cationic

lipids include saturated and unsaturated alkyl and alicyclic ethers and esters of amines, amides or derivatives thereof. Straight-chain and branched alkyl and alkene groups of cationic lipids can contain from 1 to about 25 carbon atoms. Preferred straight-chain or branched alkyl or alkene groups have six or more carbon atoms. Alicyclic groups can contain from about 6 to 30 carbon atoms. Preferred alicyclic groups include cholesterol and other steroid groups. Cationic lipids can be prepared with a variety of counterions (anions) including among others: Cl^-, Br^-, I^-, F^-, acetate, trifluoroacetate, sulfate, nitrite, and nitrate.

A well-known cationic lipid is N-[1-(2,3-dioleoyloxy)-propyl]-N,N,N-trimethylammonium chloride (DOTMA). See Felgner, P.L. et al. (1987) Proc. Natl. Acad. Sci. USA 84:7413-7417. DOTMA and the analogous diester DOTAP (1,2-bis(oleoyloxy)-3-3-(trimethylammonium)propane) are commercially available. Additional cationic lipids structurally related to DOTMA are described in U.S. Patent No. 4,897,355, which is incorporated by reference in its entirety herein.

Other useful groups of cationic lipids related to DOTMA and DOTAP are commonly called DORI-ethers or DORI-esters. DORI lipids differ from DOTMA and DOTAP in that one of the methyl groups of the trimethylammonium group is replaced with a hydroxyethyl group. The DORI lipids are similar to the Rosenthal Inhibitor (RI) of phospholipase A (Rosenthal, A.F. and Geyer, R.P. (1960) J. Biol. Chem. 235:2202-2206). The oleoyl groups of DORI lipids can be replaced with other alkyl or alkene groups, such as palmitoyl or stearoyl groups. The hydroxyl group of the DORI-type lipids can be used as a site for further functionalization, for example for esterification to amines, like carboxyspermine.

Additional cationic lipids which can be employed in the compositions and methods of this invention include those described as useful for transfection of cells in PCT application

WO 91/15501 published October 17, 1991, Pinnaduwage, P. et al. (1989) Biochem. Biophys. Acta. 985:33-37; Rose, J.K. et al. (1991) BioTechniques 10:520-525; Ito, A et al. (1990) Biochem, Intern, 22:235-241.

The polycationic lipid formed by conjugating polylysine to DOPE (Zhou, X. et al. (1991) Biochem. Biophys. Acta 1065:8-14), as well as other lipopolylysines, can also be employed in the methods and compositions of this invention.

Polycationic lipids containing carboxyspermine are also useful in the compositions and methods of this invention. Behr, J-P. et al. (1989) Proc. Natl. Acad. Sci. 86:6982-6986 and EPO published application 304 111 (1990) describe carboxyspermine-containing cationic lipids including 5-carboxyspermylglycine dioctadecyl-amide (DOGS) and dipalmitoylphosphatidylethanolamine 5-carboxyspermylamide (DPPES). Additional cationic lipids can be obtained by replacing the octadecyl and palmitoyl groups of DOGS and DPPES, respectively, with other alkyl or alkene groups.

U.S. Patent No. 5,334,761, which is incorporated by reference in its entirety herein, describes cationic lipids which are useful in this invention.

In the transfection compositions of this invention cationic lipids can optionally be combined with non-cationic lipids, preferably neutral lipids, to form lipid aggregates that bind to the modified-peptide-nucleic acid complex. Neutral lipids useful in this invention include, among many others: lecithins; phosphotidyletahnolamine; phosphatidylethanolamines, such as DOPE (dioleoylphosphatidyl-ethanolamine), POPE (palmitoyloleoyl-phosphatidylethanolamine) and distearoylphosphatidylethanolamine; phosphotidylcholine; phosphatidylcholines, such as DOPC (dioleoylphosphidylcholine), DPPC (dipalmitoylphospha-tidylcholine) POPC (palmitoyloleoyl-phosphatidylcholine) and distearoylphosphatidylcholine; phosphatidylglycerol; phosphatidylglycerols, such as DOPG (dioleoylphospha-

tidylglycerol), DPPG (dipalmitoylphosphatidyl-glycerol), and distearoylphosphatidylglycerol; phosphatidyl-serine; phosphatidylserines, such as dioleoyl- or dipalmitoyl-phospatidylserine; diphosphatidylglycerols; fatty acid esters; glycerol esters; sphingolipids; cardolipin; cerebrosides; and ceramides; and mixtures thereof. Neutral lipids also include cholesterol and other 3βOH-sterols.

The present invention is based on the discovery that certain peptides can significantly enhance the efficiency of transfection of eukaryotic cells. The compositions and methods of the invention comprise fusagenic or nuclear localization peptides which significantly improve the efficiency of transfection when bound to nucleic acid prior to adding the transfection reagent. These bound nucleic acids are more efficiently transported to the cell nucleus, thus requiring less nucleic acid starting material. Although the present invention is exemplified using a cationic lipid delivery system, fusagenic and nuclear localization peptides are effective in enhancing transfection using a variety of known delivery systems. The present invention thus contemplates the use of these peptides to enhance transfection using other delivery means including, without limitation, electroporation (T.K. Wong and E. Neumann (1982) *Biochem. Biophys. Res. Commun.* 107:584 and E. Neumann et al. (1982) *EMBO J.* 1:841), calcium phosphate (F.L. Graham and A. J. Vander Eb (1973) *Virology* 52:456), microinjection (M.R. Capecchi (1920) 22:479), ballistic transformation using microscopic particles coated with DNA (D.T. Tomes et al. (1990) *Plant Mol. Biol. Manual* A13:1-22 and G.N. Ye et al. (1990) *Plant. Molec. Biol.* 15:809) DEAE-dextran (A. Vaheri and J.S. Pagano (1965) *Science* 175:434), and polybrene-DMSO (S. Kawai and M. Nishizawa (1984) *Molec. Cell. Biol.* 4:1172).

Transfection compositions of this invention include compositions for transfecting eukaryotic cells using a peptide having a nuclear localization sequence or a fusagenic peptide crosslinked to a polycation. Peptides having a nuclear

localization sequence or fusagenic peptide crosslinked to a polycation are also a part of the invention. Preferred crosslinkers include, for example, heterobifunctional crosslinkers. The polycation is preferably a polyamine. As previously discussed, the transfection compositions and peptides of the invention are useful with a wide variety of delivery systems including, without limitation, electroporation, calcium phosphate, microinjection, ballistic transformation, DEAE-dextran and polybrene-DMSO. The present invention thus includes methods for transfecting a eukaryotic cell with a nucleic acid, the method generally comprising the steps of (1) admixing a peptide with a nucleic acid to form a peptide-nucleic acid complex; and (2) introducing the peptide-nucleic acid complex from step (1) into the cell using a known delivery means. One of ordinary skill in the art, based on knowledge generally available to the art including the present disclosure, can use the compositions and peptides of the present invention with any delivery system without the expense of undue experimentation.

It will be readily apparent to those of ordinary skill in the art that a number of parameters are important for optimal transfection. These parameters include cationic lipid concentration, relative amounts of cationic and non-cationic lipid, the concentration of nucleic acid, the medium employed for transfection, the length of time the cells are incubated with transfection composition, the amount of viral peptide employed, the amount of DNA-binding group or polyamine employed, and the way in which the components of the transfection composition are combined. It may be necessary to optimize these parameters for each cell type to be transfected. Such optimization is routine employing the guidance provided herein and transfection assays as described in the Examples herein.

It will also be apparent to those of ordinary skill in the art that alternative methods, reagents, procedures and techniques other than those specifically detailed herein can be employed or readily adapted to produce the transfection compositions of this

invention and practice the transfection methods of this invention. Such alternative methods, reagents, procedures and techniques are within the spirit and scope of this invention.

The transfection compositions and methods of this invention are further illustrated in the following non-limiting Examples. All abbreviations used herein are standard abbreviations in the art. Specific procedures not described in detail in the Examples are well-known in the art.

EXAMPLES

Example 1: Cell Cultures and Plasmids

Human primary fibroblasts (HPF) were isolated from neonatal foreskin dermis and prepared as described in Hawley-Nelson, P., et al. (1993) Focus 15:73, incorporated by reference herein, and cultured for up to 20 passages. Baby hamster kidney (BHK-21) cells were obtained from the American Type Culture Collection (Rockville, MD). Both cultures were grown in Dulbecco's-modified Eagle's medium (DMEM) containing 10% (v/v) fetal bovine serum (FBS), 100 U/ml penicillin (PEN) and 100 mg/ml streptomycin (STREP). Cultures were passaged at confluence using 0.25% (v/v) trypsin, 0.1 mM EDTA. All culture reagents were from Gibco/BRL (Life Technologies, Inc., Gaithersburg, MD).

The plasmid vector pCMVßgal is a commercially available (Clontech, CA) mammalian reporter vector containing the E. coli ß-galactosidase (ß-gal) gene under the control of the Cytomegalovirus promoter. See: MacGregor et al. (1989) Nucleic Acids Res. 17: 2365; Norton et al. (1985) Mol. and Cell Biol. 5:281; Alam (1990) Anal. Biochem. 188:245. Plasmid DNA was purified by standard cesium chloride methods.

Example 2: Peptides and Peptide-Spermine Conjugates

Peptides were synthesized using automated solid phase peptide synthesis as described, e.g., in Stewart et al. (1984) Solid Phase Peptide Synthesis, Pierce Chemical Company, Rockford, Illinois. Peptides were synthesized using a polyamide-kieselguhr composite resin and a MilliGen 9050 peptide synthesizer (MilliGen/Biosearch, Burlington, MA); coupling cycles were performed according to the manufacturer's recommendations including the following: 9-fluorenyl-methyloxy-carbonyl (Fmoc) amino acid is activated as pentafluorophenyl ester (-OPfp ester), deprotect alpha-amino groups by 20% piperidine in N,N-dimethylformamide (DMF), cleave peptide from resin and deprotect by 95% trifluoroacetic acid (TFA), precipitate and wash crude peptide with ether. Peptides were purified by high pressure liquid chromatography on a Vy-Dac C(18) reverse-phase column using a Waters system. The mobile phase consisted of a gradient from 0.01% TFA in 95% water/acetonitrile to 0.01% TFA in 25% water/acetonitrile. Peptide sequences are shown in Tables 1 and 2.

Peptide-spermine conjugates were prepared using a heterobifunctional cross-linking agent sulfo-SMPB (Pierce Chemical Co., Rockford, IL). Briefly, 100 mg/ml sulfo-SMPB in DMF was diluted to 20 mg/ml using 50 mM sodium phosphate buffer (pH 7.5). 50 mg/ml spermine in 50 mM sodium phosphate buffer was then added to the sulfo-SMPB solution at a 3:1 molar ratio. After 1 hour at room temperature, the reaction mixture was fractionated (LH-20 column) using the sodium phosphate buffer. The first major peak (spermine-MPB) was collected and mixed at a 1:1.5 to 1:2 ratio with synthetic peptide, either in pure powder form or in acetonitrile/water solution. Excess peptide was separated on a LH-20 column eluted with water. The peptide-spermine conjugate was stored frozen until use.

Example 3: Transfection of Human Primary Fibroblast and BHK-21 Cells

For transfection, cells were trypsinized the day prior to treatment and replated at 8 X 10^4 or 1 X 10^5 (human fibroblasts)

or 2 X 10^4 (BHK-21) cells per well in 24-well plates. Cells were rinsed once with serum-free DMEM prior to transfection and 0.25 ml serum-free DMEM was added to each well. Plasmid DNA was diluted to 10 mg/ml in Opti-MEM™-I (Gibco/BRL: Life Technologies, Inc., Gaithersburg, MD) medium and allowed to bind to or complex with various concentrations of enhancing peptides (7-470 μM) for 30 minutes at room temperature. LIPOFECTAMINE™ (Gibco/BRL: Life Technologies, Inc., Gaithersburg, MD) was separately diluted in Opti-MEM™-I (40-400 μg/ml) and allowed to incubate for 5 minutes at room temperature. LIPOFECTAMINE™ is a 3:1 (w/w) mixture of the polycationic lipid, 2,3-dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate (DOSPA), and DOPE. After this pre-incubation period, the diluted peptide-DNA complex mixture was gently mixed with an equal volume of the diluted transfection reagent. The reaction mixture was incubated at room temperature for 30 minutes to form peptide-DNA-lipid aggregates. The aggregates were then diluted 6-fold with serum-free DMEM and 0.3 ml diluted aggregate was added to each well. Cells were incubated at 37°C. After approximately 5 hours, one ml growth medium (DMEM containing 13% FBS) was added to each well. Cells were assayed the next day for ß-galactosidase activity.

Example 4: Transient Transfection Assays

In situ staining was used to demonstrate ß-galactosidase expression (Sanes, J.R. et al. (1986) EMBO J. 5:3133). Cells were rinsed with PBS, fixed for 5 min in 2% (v/v) formaldehyde, 0.2% glutaraldehyde in PBS, rinsed twice with PBS, and stained overnight with 0.1% X-gal (Gibco/BRL: Life Technologies, Inc., Gaithersburg, MD), 5 mM potassium ferrocyanide, 5 mM potassium ferrocyanide, 2mM $MgCl_2$ in PBS. Rinsed cells were photographed using a 10X objective on a Nikon inverted microscope with Hoffman optics. Transfection efficiency is evaluated by counting or estimating the number of ß-gal positive (blue-stained) cells.

Enzyme activity of lysed cell extracts was used to compare levels of expression resulting from different treatment protocols. One to two days following transfection, cells were rinsed once with PBS and frozen at -70°C in 0.15-0.25 ml/well 0.1% Triton X-100 (Sigma Chemical Co., St. Louis, MO) and 0.1M Tris, pH 8.0. After rapid thawing at 37°C, the lysate was cleared by centrifugation. Lysed cell extracts were assayed for ß-galactosidase activity employing the method essentially as described in Sambrook et al. (1989) Molecular Cloning A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, p. 16.66. Briefly, soluble cell extract containing 2-6 µg protein was added to 100 µl 0.1M sodium phosphate buffer (pH 7.5) containing 1 mM $MgCl_2$, 50 mM ß-mercaptoethanol and 0.88 mg/ml σ-nitrophenyl-ß-D-galacatopyranoside (ONPG) in a 96-well microtiter plate. A standard curve of 10-70 ng ß-galactosidase (Gibco/BRL: Life Technologies, Inc., Gaithersburg, MD) was included on the plate. Yellow color developed in 5-20 minutes at 37°C. The reaction was stopped by adding 150 µl 1 M Na_3CO_2; OD_{420} was determined on a microtiter plate reader.

Example 5: Transfection of Human Fibroblast Cells using Cationic Lipids and Viral Peptides

The following viral peptides were synthesized using automated solid phase peptide synthesis as described in Example 2: the membrane fusion regions of influenza virus hemagglutinin, peptides E5 and K5 (see Kamata, H. et al. (1994) Nucleic Acids Res. 22:536-537); hemagglutinin peptide, HApep (see Epand et al. (1992) Biopolymers 32:309); vesicular stomatitis virus G-protein, VSVG (see Schlegel, R. and Wade, M. (1985) J. Virol. 53:319); and the nuclear localization signal of SV40 large T antigen, NLS (see Lanford et al. (1986) Cell 46:575).

Human fibroblasts were plated the day before transfection at 8 X 10^4 per well on a 24-well dish. Before transfection, the cells were rinsed with serum-free DMEM. Two 25 µl aliquots of Opti-MEM™ I medium, one containing 3 µg LIPOFECTAMINE™ and the

other containing 0.2 μg pCMVßgal DNA, were combined to form complexes for 30 min at room temperature. Peptides were dissolved in dimethylsulfoxide (DMSO) at 250 X the final% %*^U

ration (see Table 1). 1 μl peptide solution was added to 250 μl serum-free DMEM transfection medium and added to the rinsed cells. For the combined treatments, E5, K5 and VSVG were all used at 5 μM concentrations. The DNA-lipid aggregates in Opti-MEM™ I were then added to the transfection medium on the cells. After 24 hours incubation at 37°C, cells were harvested, extracted and assayed for ß-galactosidase activity as described in Example 4.

As shown in Figure 1, the optimal concentrations of peptides produced a 2-3-fold enhancement of transfection activity with LIPOFECTAMINE™ in human fibroblasts.

Table 1: Viral peptides tested for enhancement of LIPOFECTAMINE™ transfections.

Peptide	Sequence	Concentration (μM)	(SEQ ID NO.: 1)
E5	GLFEAIAEFIEGGWEGLIEG	0.1	1
K5	GLFKAIAKFIKGGWKGLIKG	5	2
HApep	GLFGAIAGFIENGWEGMIDG	10	3
VSVG	KFTIVF	1	4
NLS	GYGPKKKRKVGG	NA	5

Example 6: Transfection of Human Fibroblast Cells using Cationic Lipids and Spermine-Peptide Conjugates

The NLS and VSVG peptides (Example 5) were synthesized with cysteine in the N- or C- terminal position, then conjugated to spermine as described in Example 2. These spermine-peptide conjugates were then evaluated to determine the effect of covalently bound spermine on the efficiency of transfection. Human fibroblasts were plated the day before transfection at 8 x 10^4 per well on a 24-well plate. Before transfection, the cells were rinsed with serum-free DMEM. Aqueous solutions of 0.15 mM spermine-modified peptides were prepared, each diluted to 50 μM concentrations using Opti-MEM™ containing 6 μg/ml DNA, then incubated at room temperature. After 15 minutes, an equal volume of OptiMEM™ containing 120 μg/ml LIPOFECTAMINE™ was added, and the mixtures were incubated for 30 minutes to allow complex formation. These complexes in OptiMEM™ were diluted 6-fold with serum-free DMEM, and 0.3 ml of diluted complex was added to each well. After approximately 5 hours incubation at 37°C, one ml DMEM containing 10% (v/v) FBS was added to each

well. The next day cells were fixed and stained with X-gal, as described in Example 4. The level of expression was estimated by observation and reported as fold enhancement of peak LIPOFECTAMINE™ transfection levels without peptide. The results are shown in Table 2. As shown in Table 2, covalent binding of spermine to NLS and VSVG peptides causes a significant enhancement in the efficiency of cationic lipid transfection.

Table 2. Spermine-modified peptide enhancement of LIPOFECTAMINE™ transfections.

Peptide	Sequence	Fold Enhancement	SEQ ID NO:
NLS-sp	GYGPKKKRKVGGCsp	1-2	6
Short NLS-sp	PKKKRKVGGCsp	1	7
sp-NLS	spCGYGPKKKRKVGG	5-10	8
VSVGsp	KFTIVFCsp	3-5	9

The spermine-NLS and spermine-VSVG conjugates were further tested to determine the optimal concentration for enhancement. Human fibroblasts were plated the day before transfection at 8 X 10^4 per well on a 24-well dish. Before transfection, the cells were rinsed with serum-free DMEM. Two 25 ml aliquots of OptiMEM™ medium were prepared; one contained 3 μg LIPOFECTAMINE™ and the second contained 0.2 μg pCMVßgal DNA. Spermine-peptide conjugates were dissolved in water at 25-fold their final concentration. 10 μl spermine-peptide solution was added to the OptiMEM™-DNA aliquot and incubated for 15 minutes at room temperature. The LIPOFECTAMINE™ solution was then added to the DNA-peptide solution, and the mixture incubated 30 minutes at room temperature to allow complex formation. The complex solution was diluted to 0.3 ml with serum-free DMEM and added to each well. After approximately 5 hours incubation at 37°C, one ml DMEM containing 10% (v/v) FBS was added to each well. The next day cells were harvested, lysed and lysed cell extracts were

assayed for ß-galactosidase activity, as described in Example 4. The results as shown in Figure 2. As shown in Figure 2, 5 to 17 μM final concentration of spermine-modified peptide in the transfection medium (5-10-fold higher concentration in the pre-incubation with DNA) was found to be optimal.

Example 7: Comparison of Peptides, Spermine-Peptide Conjugates and Spermine/Peptide Mixtures on Transfection of Human Primary Fibroblast Cells

The NLS and VSVG peptides (Example 5) were synthesized with cysteine in the N- or C- terminal position, then conjugated to spermine as described in Example 2. These spermine-peptide conjugates were then evaluated for enhancement of transfection relative to the corresponding spermine/peptide mixture. Human fibroblasts were plated the day before transfection at 8 X 10^4 per well on a 24-well dish. Before transfection, cells were rinsed with serum-free DMEM. Two 25 μl aliquots of Opti-MEM™ I medium were prepared, one containing 3 μg LIPOFECTAMINE™ and the second containing 0.2 μg pCMVßgal DNA. Spermine-peptide conjugates or mixtures of peptides with and without spermine were dissolved in water at 25-fold their final concentration. 10 μl peptide was added to the OptiMEM™-DNA aliquot and incubated for 15 minutes at room temperature. The LIPOFECTAMINE solution was added to the DNA-peptide solution, and incubated 30 minutes at room temperature to allow complex formation. The complex solution was diluted to 0.3 ml with serum-free DMEM and added to each well. After approximately 5 hours incubation at 37°C, one ml DMEM containing 10% (v/v) FBS was added to each well. The next day cells were harvested, lysed and lysed cell extracts were assayed for ß-galactosidase activity, as described in Example 4. The results as shown in Figure 3. As shown in Figure 3, the addition of spermine to the unconjugated peptides does not enhance transfection. Surprisingly, the addition of the NLS peptide without the terminal spermine residue, when allowed to complex with the DNA before addition of LIPOFECTAMINE™, enhanced transfection as much or more than the spermine-NLS conjugate.

Example 8: Effect of Order of Reagents on Transfection of Human Primary Fibroblast Cells

The spermine-NLS and spermine-VSVG peptides were synthesized as described in Example 2. Transfection protocols were as described in Example 3, except that the order of addition of reagents was varied to test the effect on transfection of complex formation between the spermine-modified peptide and DNA. Results are shown in Figure 4.

Human fibroblasts were plated the day before transfection at 8 X 10^4 per well on a 24-well dish. Before transfection, cells were rinsed with serum-free DMEM. Two 25 µl aliquots of Opti-MEM™ I medium were prepared, one containing 3 µg LIPOFECTAMINE™ and the other containing 0.2 µg pCMVßgal DNA. To form spermine-modified-peptide-DNA complexes, spermine-peptide conjugates were dissolved in water at 25-fold their final concentration, then 10 µl peptide solution was added to the OptiMEM™-DNA aliquot and incubated for 15 minutes at room temperature. To test the effect of order of addition on reagents, the peptides were first added to the diluted LIPOFECTAMINE™ in OptiMEM™ and incubated 15 minutes at room temperature before mixing with the DNA solution. The LIPOFECTAMINE™/peptide solution was then added to the DNA solution and incubated 30 minutes at room temperature to allow lipid-DNA complex formation. The complex solution was diluted to 0.3 ml with serum-free DMEM and added to each well. After approximately 5 hours incubation at 37°C, one ml DMEM containing 10% (v/v) FBS was added to each well. The next day cells were harvested, lysed and lysed cell extracts were assayed for ß-galactosidase activity, as described in Example 4. The results as shown in Figure 4. As shown in Figure 4, the addition of the spermine-peptide conjugate to the transfection lipid prior to complexing with DNA significantly reduces the efficiency of transfection.

Example 9: Transfection of Human Primary Fibroblast Cells Using Spermine-Modified-Peptide or Poly-L-Lysine and Cationic Lipids

The spermine-NLS peptide was synthesized as described in Example 2. Poly-L-lysine (MW 5200) was obtained from Sigma Chemical Co. (St. Louis, MO). Transfection protocols were as described in Example 3, except that poly-L-lysine was used to compare the effect of a poly-amino acid/DNA complex with the effect of the spermine-peptide-DNA complex on transfection. Human fibroblasts were plated the day before transfection at 8 X 10^4 per well on a 24-well dish. Before transfection, cells were rinsed with serum-free DMEM. Two 25 µl aliquots of Opti-MEM™ I medium were prepared, one containing 3 µg LIPOFECTAMINE™ and the other containing 0.2 µg pCMVßgal DNA. Spermine-conjugated NLS and poly-L-lysine were dissolved in water at 25-fold their final concentration. 10 µl peptide was added to the OptiMEM-DNA aliquot and incubated for 15 minutes at room temperature. The LIPOFECTAMINE™ solution was added to the DNA-peptide solution, and incubated 30 minutes at room temperature to allow lipid-DNA-peptide complex formation. The complex solution was diluted to 0.3 ml with serum-free DMEM and added to each well. After approximately 5 hours incubation at 37°C, one ml DMEM containing 10% (v/v) FBS was added to each well. The next day cells were harvested, lysed and lysed cell extracts were assayed for ß-galactosidase activity, as described in Example 4. The results as shown in Figure 5. As shown in Figure 5, complex formation of 0.1mM poly-L-lysine (MW5200) with the DNA prior to formation of complexes with lipid transfection reagents enhanced transfection at least as much as the spermine-NLS precomplexed to the DNA.

Example 10: Transfection of BHK-21 Cells Using Spermine-NLS Conjugate and Cationic Lipids

The spermine-NLS peptide was synthesized as described in Example 2. Transfection protocols were as described in Example 3, except that the concentration of the complexes was varied.

BHK-21 cells were plated the day before transfection at 2 X 10^4 per well on a 24-well dish. Before transfection, cells were rinsed with serum-free DMEM. Each well was fed with 0.25 ml serum-free DMEM transfection medium. Two 25 µl aliquots of Opti-MEM™ medium were prepared, one containing 3 µg LIPOFECTAMINE™ and the second containing 0.4 µg pCMVßgal DNA. Spermine-peptide conjugates were dissolved in water at 25-fold their final concentration. 10 µl peptide was added to the OptiMEM™-DNA aliquot and incubated for 15 minutes at room temperature. The LIPOFECTAMINE™ solution was added to the DNA-peptide solution, and incubated 30 minutes at room temperature to allow complex formation. 50 µl of full-strength complexes and complexes diluted 1:4 and 1:10 were added to each well. After approximately 5 hours incubation at 37°C, one ml DMEM containing 13% (v/v) FBS was added. The next day cells were harvested, lysed and lysed cell extracts were assayed for ß-galactosidase activity, as described in Example 4. The results are shown in Figure 6. Figure 6 shows the peak expression levels over a dose-response range for the diluted complexes. As shown in Figure 6, pre-complexing spermine-NLS to DNA prior to formation of complexes with the lipid transfection reagent allows enhanced transfection with less total DNA.

Example 11: Transfection of BHK-21 Cells Using Spermine-VSVG Conjugate and Cationic Lipids

The spermine-VSVG peptide was synthesized as described in Example 2. Transfection protocols were as described in Example 3, except that the concentration of the complexes was varied. BHK-21 cells were plated the day before transfection at 2 X 10^4 per well on a 24-well dish. Before transfection, cells were rinsed with serum-free DMEM. Each well was fed with 0.25 ml serum-free transfection medium. Two 25 µl aliquots of Opti-MEM™ medium were prepared, one containing 3 µg LIPOFECTAMINE™ and the second containing 0.4 µg pCMVßgal DNA. Spermine-VSVG conjugates were dissolved in water at 25-fold their final concentration. 20 µl peptide was added to the OptiMEM™-DNA aliquot and

incubated for 15 minutes at room temperature. The LIPOFECTAMINE™ solution was added to the DNA-peptide solution, and incubated 30 minutes at room temperature to allow complex formation. 50 µl of full-strength complexes and complexes diluted 1:4 and 1:10 were added to each well. After approximately 5 hours incubation at 37°C, one ml DMEM containing 10% (v/v) FBS was added. The next day cells were harvested, lysed and lysed cell extracts were assayed for ß-galactosidase activity, as described in Example 4. The results are shown in Figure 7. Figure 7 shows the peak expression levels over a dose-response range for the diluted complexes. As shown in Figure 7, pre-complexing spermine-VSVG to DNA prior to formation of complexes with the lipid transfection reagent allows enhanced transfection with less total DNA.